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**BEFORE THE BOARD OF PATENT APPEALS
AND INTERFERENCES**

Application Number: 10/561,826
Filing Date: October 17, 2006
Appellant(s): VERFAILLIE ET AL.

Anne Brown
For Appellant

EXAMINER'S ANSWER

This is in response to the appeal brief filed May 23, 2001, appealing from the Office action mailed June 22, 2010.

(1) Real Party in Interest

The examiner has no comment on the statement, or lack of statement, identifying by name the real party in interest in the brief.

(2) Related Appeals and Interferences

The examiner is not aware of any related appeals, interferences, or judicial proceedings which will directly affect or be directly affected by or have a bearing on the Board's decision in the pending appeal.

(3) Status of Claims

The following is a list of claims that are rejected and pending in the application:

Claims 2-4 and 7-12 are canceled. Claims 7-11 were rejected in the final office action but were canceled in Appeal brief. Claims 1, 5, 6 and 13 are pending and rejected.

(4) Status of Amendments After Final

The examiner has no comment on the appellant's statement of the status of amendments after final rejection contained in the brief.

(5) Summary of Claimed Subject Matter

The examiner has no comment on the summary of claimed subject matter contained in the brief.

(6) Grounds of Rejection to be Reviewed on Appeal

The examiner has no comment on the appellant's statement of the grounds of rejection to be reviewed on appeal. Every ground of rejection set forth in the Office action from which the appeal is taken (as modified by any advisory actions) is being maintained by the examiner except for the grounds of rejection (if any) listed under the subheading "WITHDRAWN REJECTIONS." New grounds of rejection (if any) are provided under the subheading "NEW GROUNDS OF REJECTION."

(7) Claims Appendix

The examiner has no comment on the copy of the appealed claims contained in the Appendix to the appellant's brief.

(8) Evidence Relied Upon

- A. WO02/086073, Studer et al, published on Oct 31, 2002).
- B. US2003/0211605, Lee et al., published on Nov 13, 2003, priority May 1, 2000.
- C. Walsh et al. Neuronal Survival and neurite extension supported by astrocytes cocultured in transwells. Neuroscience Letter. 1992 Apr 13; 138: 103-106, Abstract.
- D. US5851832, Weiss et al., 1998-12. (Note that this reference is not a new rejection. It is cited to support the examiner's rebuttal that the procedures of co-culturing neural cells with astrocytes are known in the art, and are used as

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standard protocols for neuronal cultures to enhance neuronal survival and differentiation).

(9) Grounds of Rejection

The following ground(s) of rejection are applicable to the appealed claims:

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

(a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negated by the manner in which the invention was made.

The factual inquiries set forth in *Graham v. John Deere Co.*, 383 U.S. 1, 148 USPQ 459 (1966), that are applied for establishing a background for determining obviousness under 35 U.S.C. 103(a) are summarized as follows:

1. Determining the scope and contents of the prior art.
2. Ascertaining the differences between the prior art and the claims at issue.
3. Resolving the level of ordinary skill in the pertinent art.
4. Considering objective evidence present in the application indicating obviousness or nonobviousness.

A. Claims 1, 5, 6 and 13 are rejected under 35 U.S.C. 103(a) as being unpatentable over WO02/086073 (Studer et al., published Oct 31, 2002, cited in office action mailed 10/18/07) in view of US2003/0211605 (Lee et al., published Nov 13, 2003, priority May 1, 2000).

Claims 1, 5, 6 and 13 are drawn to a method for inducing stem cells to differentiate into neuronal cells comprising

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- a) culturing said stem cells with bFGF (basic fibroblast growth factor);
- b) culturing the cells of step a) with FGF8 (fibroblast growth factor 8) and SHH (Sonic Hedgehog);
- c) culturing the cells of step b) with BDNF (brain-derived neurotrophic factor); and
- d) co-culturing the cells of step c) with astrocytes, wherein the cells are cultured according to steps a) through d) for at least seven days at each step.

Dependent claims 5 and 6 are directed to mammalian stem cells (claim 5) and human stem cells (claim 6). Note that the instant invention do not claim a method to induce differentiation of stem cells into specific neuronal populations or to induce differentiation of stem cells into different ratios of neuronal cells.

Studer (WO02086073) teaches a method of inducing stem cells to differentiate into neuronal cells comprising culturing embryonic stem cells in the presence of bFGF, FGF8, Shh, BDNF and co-culturing the cells with astrocytes as in instant claims 1, 5, 6 and 13 (see p. 4, paragraph 12-p. 6 paragraph 20; p. 24, example 2-p. 30, in particular). Studer teaches a method of expanding embryonic stem (ES) cells isolated from embryonic or adult tissues in a DMEM+F12 medium comprising bFGF, FGF8, Shh, BDNF, N2 supplement, insulin, transferrin, selenite and fibronectin (see p. 25-26, paragraphs 75-78, in particular). Studer teaches that in order to generate astrocytes, before adding SHH and FGF8 into the culture medium, the ES cells are cultured and proliferate in a culture medium in the presence of bFGF (see p. 5, paragraphs 16-17; p. 26, paragraph 78, in particular). Studer teaches that in order to enhance generation of

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dopaminergic and serotonergic neurons, ES cells are cultured in a proliferation culture medium in the presence of FGF8 and SHH for 6-9 days before induction of neuronal differentiation (see p. 28, paragraph 85; p. 29, paragraph 86, in particular). Studer also teaches that the expanded cultured ES cells from stage IV (enhanced proliferation of neuronal stem cells) are induced to differentiate into neurons in the culture medium in the presence of BDNF and in the absence of SHH and FGF8 for 4-10 days (see p. 26, paragraph, 78; p.29, paragraph 87, in particular). But Studer does not explicitly teach adding bFGF, FGF8, SHH, BDNF sequentially, does not explicitly teach co-culturing with astrocytes and does not explicitly teach that cells were cultured for at least 7 days at each step.

Although the instant method recites adding bFGF, FGF8, SHH, BDNF sequentially, at the end of the final steps, the culture medium contains identical growth factors as those in the Studer's method to induce neuronal differentiation. The result of inducing neuronal differentiation from stem cells by adding bFGF, FGF8, SHH and BDNF in a different order are expected (i.e. induction of neuronal differentiation), and do not result in any unexpected result because at the end of the culture, the culture medium still contains the same growth factors (bFGF, FGF8, SHH and BDNF) added in the culture, the same cultured ES cells, and the cultured ES cells are capable of differentiate into neurons. Note that the instant invention do not claim a method to induce differentiation of stem cells into specific neuronal populations or to induce differentiation of stem cells into different ratios of specific neuronal cells.

In addition, Studer teaches differentiation of cultured embryonic stem cells into neurons and glial cells, which include astrocytes. The presence of culturing neurons together with glial cells including astrocytes meets the limitation "co-culturing with astrocytes" as recited in instant claims 1 and 13 (see p. 25-26, paragraphs 75-78, in particular). Although Studer does not specify glial cells in the culture, the glial cells encompass astrocytes as supported by Lee (see below). Although Studer does not explicitly teach co-culturing stem cells that are treated with the claimed growth factors with astrocytes, the cell culture of Studer is a cell mixture, which encompasses astrocytes. Thus, the presence of glial cells including astrocytes in the culture of stem cells would meet the limitation "co-culturing cells with astrocytes". In addition, it is known in the art that neuronal cultures require astrocytes to maintain and support neuronal survival and differentiation because the mixture of neuronal stem or progenitor cells that are capable of differentiating into neurons also encompass cells that are capable of developing or differentiating into astrocytes. Further, it is known in the art and it is a standard procedure to culture neuronal progenitor cells or neural stem cells with astrocytes or an astrocyte feeder layer to maintain or enhance neuronal survival as evidenced by Walsh et al. (Neurosci. Lett 1992. 138: 103, abstract, cited in office action dated 12/24/08, p. 8) and US5851832 (Weiss et al, see col.30, lines 32-45; col. 37, lines 29-42, example 7, in particular). Thus, it is obvious to co-culture stem cells that have been treated with bFGF, FGF8, Shh and BDNF with astrocytes to induce neuronal differentiation.

In addition, although Studer does not explicitly teach at least 7 days for each step as recited in instant claims 1 and 13, each step and each stage of the culture conditions of Studer require 6-9 days and the whole culture procedures take more than one month (see p.27-29, in particular). Thus, the culturing steps and conditions are within the limitation of the instant claims 1 and 13. Studer also teaches that the cultured stem cells are mammalian stem cells, human stem cells, neural stem cells, embryonic stem cells, embryonic germ cells and brain as recited in instant claims 5-6 (see p. 12, paragraph 49; p. 19 paragraphs 63-p.20). Studer also teaches differentiation of stem cells into dopaminergic, serotonergic, GABAergic neurons and combinations thereof (see p.12, paragraph 48; p17, paragraph 59, in particular).

Lee (US2003/0211605) teaches a method of inducing stem cells to differentiate into neuronal cells comprising culturing embryonic stem cells in the presence of bFGF, FGF8, Shh, and co-culturing the cells with astrocytes as in instant claims 1, 5, 6 and 13 (see abstract; p. 9, [0116]-p. 10, [0129]; p. 2, [0016]-[0021]; p.3, [0040]-[0043]; p.4, [0045]-[0048], [0058]; p. 5, [0071]-p.7,[0093];p.11 [0147]-[0148]; p. 13, example 1-p. 16, example 7, in particular). Lee teaches a method of expanding embryonic stem (ES) cells and the CNS precursor cells in a DMEM plus F12 medium comprising bFGF, FGF8, Shh, N2 supplement, insulin, transferrin, selenite, putrescine, and progesterone as recited in instant claims 1 and 13 (see p.9, [0123]-[0126], in particular). Lee teaches differentiation of cultured embryonic stem cells into neurons using the same medium in presence of ascorbic acid, and the differentiation of stem cells encompasses 3% astrocytes. The presence of astrocytes in the culture of stem cells that are differentiated

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into neurons meets the limitation of "comprising co-culture astrocytes" as recited in instant claims 1 and 13.

Lee teaches that the CNS precursor cells (including CNS stem cells and embryoid cells/neurospheres) are expanded in the CNS proliferation medium in the presence of bFGF or EGF for about 6 to 7 days (see p. 9, [0123]-[0124], in particular). Lee also teaches that the culture medium may also be supplemented with SHH and FGF8 because SHH and FGF8 are more effective to increase the generation of dopaminergic neurons (see p.9, [0125], in particular). Moreover, Lee teaches that neuronal differentiation is induced by withdrawal of at least one neurological agent, such as bFGF in the culture medium in the presence of the factors to enhance the generation of dopaminergic neurons for five to 6 days (see p. 9, [0128]; p. 14, example 5, in particular).

Although Lee does not explicitly teach at least 7 days for each step as recited in instant claims 1 and 13, the complete culture procedures of Lee take more than one month (see p.8, [0111], in particular). Lee also teaches that the cultured stem cells are mammalian stem cells, human stem cells, neural stem cells, embryonic stem cells, embryonic germ cells and brain as recited in instant claims 5-6 (see p. 4, [0051], in particular). Lee also teaches differentiation of stem cells into dopaminergic, serotonergic, GABAergic neurons and combinations thereof (see p.9, [0125]-p.10,[0127], in particular). Lee also teaches BDNF to promote survival and function of neurons and glial cells ([0070]-[0071], in particular) or cells transfected with BDNF gene to promote survival and function of neurons ([0177], in particular).

It would have been obvious to one of ordinary skill in the art at the time the instant invention was made to incorporate the teachings of Lee to the method of Studer to optimize the culture conditions to induce neuronal differentiation from stem cells. The person of ordinary skill in the art would have been motivated to do so with an expectation of success because both Lee and Studer teach the use of different growth factors separately and the use of different growth factors can enhance different cell populations at different stages. For example, Lee and Studer teach that bFGF can be used to expand ES cells, and FGF8 and SHH can be used to increase the generation of dopaminergic neurons. In addition, the culture medium in the presence of bFGF and in the absence of FGF8 and SHH can increase astrocyte generation. Further, the culture medium in the presence of BDNF and in the absence of FGF8 and SHH can induce neuronal differentiation. Thus, it would have been obvious to add different growth factors sequentially. The person would have been motivated to do so with an expectation of success because each different growth factor can increase specific type cell populations and the use of the combination of the claimed growth factors have been shown to successfully induce neuronal differentiation from stem cells. The combined teachings of Studer and Lee has taught that the addition of bFGF is to induce ES cell expansion and can enhance astrocyte generation for neuronal survival, the addition of FGF8 and SHH to the expanded ES cells after the exposure of bFGF can enhance the generation of dopaminergic and serotonergic neurons, and the addition of BDNF after the exposure of FGF8 and SHH can induce neuronal differentiation.

Moreover, although Studer and Lee do not explicitly teach at least 7 days for each step, the claimed procedures and incubation time for each step are obvious over the cited reference because the incubation time for each step is within or overlaps with the claimed incubation time. In addition, as mentioned above, it is known in the art that neural cells (neural progenitor/stem cells) co-cultured with astrocytes can enhance neuronal survival and differentiation. Thus, it has been obvious to combine the teachings of Studer and Lee to achieve and practice the claimed invention because the results of dopaminergic and serotonergic neuronal differentiation are expected. Note that

In the case where the claimed ranges "overlap or lie inside ranges disclosed by the prior art" a *prima facie* case of obviousness exists. *In re Wertheim*, 541 F.2d 257, 191 USPQ 90 (CCPA 1976); *In re Woodruff*, 919 F.2d 1575, 16 USPQ2d 1934 (Fed. Cir. 1990), See MPEP 2144.05-I.

"a *prima facie* case of obviousness exists where the claimed ranges and prior art ranges do not overlap but are close enough that one skilled in the art would have expected them to have the same properties. *Titanium Metals Corp. of America v. Banner*, 778 F.2d 775, 227 USPQ 773 (Fed. Cir. 1985)" See MPEP 2144.05-I.

"[W]here the general conditions of a claim are disclosed in the prior art, it is not inventive to discover the optimum or workable ranges by routine experimentation." *In re Aller*, 220 F.2d 454, 456, 105 USPQ 233, 235 (CCPA 1955)" See MPEP 2144.05-II.

B. Claims 1, 5, 6 and 13 are rejected under 35 U.S.C. 103(a) as being unpatentable over WO02/086073 (Studer et al., published Oct 31, 2002, cited in office action mailed 10/18/07) in view of US2003/0211605 (Lee et al., published Nov 13, 2003, priority May 1, 2000) and further in view of Song et al. (Methods in Mol. Biol. 2002. 198: 79-88).

WO02/086073 and US2003/0211605 are as set forth above but do not teach different progenitor/stem cells from different tissue to differentiate into neurons.

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Song et al. teach a method of culturing and differentiating cells from bone marrow and umbilical cord blood into neural progenitor cells and neurons in a DMEM/F12 medium comprising FGF-2/bFGF, EGF, transferrin, insulin, putrescine, progesterone, selenium, trans-retinoic acid, BDNF and NGF (see p. 80, in particular). Song et al. also teach culturing human and mouse bone marrow and human umbilical cord cultures (p. 82-83, in particular). The bone marrow and umbilical cord blood cells encompass stem cells; and nonhematopoietic progenitor cells from bone marrow are mesenchymal stem cells or bone marrow stromal cells as taught by Song et al. (p.79, in particular). Thus, Song teaches differentiation of stem cells derived from different multipotent adult progenitor cells (MAPCs) and bone marrow. It would have been obvious to one of ordinary skill in the art at the time the invention was made to differentiate stem cells that are derived from multipotent adult progenitor cells (MAPCs) and bone marrow into neurons by using the culture conditions of Studer and Lee. The person of ordinary skill in the art would have been motivated to do so with an expectation of success because multipotent adult progenitor cells (MAPCs) and cells from bone marrow have been shown to encompass stem cells that are capable of being induced to differentiate into neurons as taught by Song et al.; and Studer and Lee have taught that stem cells can be induced to differentiate into neurons in the claimed culture conditions.

(10) Response to Argument

A.

On p. 9-10 of the appeal brief, Appellants summarize what Studer and Lee disclose. In particular, Appellants state that there are five stages in differentiation protocols disclosed by Studer and Lee. Appellants argue that the first three stages do not require any of the claimed factors; a mitogen is added in stage IV, and the mitogen is withdrawn in stage V to induce neuronal differentiation. Appellants state that a mitogen factor bFGF is added in stage IV; and Shh and FGF8 are added together with bFGF to increase the ratio of dopaminergic to serotonergic neurons. Appellants' arguments have been considered but they are not persuasive.

In response, note that the stem cells used in the claimed method are not limited to any specific phenotypes of stem cells or specific types or stages of stem cells. Even if the culture protocols listed in Appellants' appendix tables are correct, Appellants cannot refer the stages described in Studer or Lee accordingly as the steps (a)-(d) cited in instant claims. The steps (a)-(d) of the instant claims can start from the stage IV as listed in Appellants' appendix. In fact, contrary to Applicant's arguments, Lee and Studer teach that bFGF can be used to expand ES cells, and FGF8 and SHH can be used to increase the generation of dopaminergic neurons. In addition, Lee and Studer teach that the culture medium in the presence of bFGF and in the absence of FGF8 and SHH can increase astrocyte generation. Further, Lee and Studer teach that the culture medium in the presence of BDNF and in the absence of FGF8 and SHH can induce neuronal

differentiation. Thus, it is obvious to add growth factors sequentially based on the desired cell populations to increase or induce specific neuronal cell populations.

On p. 11-13 of the appeal brief, Appellants summarize different office actions. In particular, Appellants state that the examiner's rationale appeared to have supported a rejection on the ground of anticipation. On p. 13 of the appeal brief, Appellants argue that neither Studer nor Lee anticipates the claims. Appellants argue that no discussion was provided in the office action dated 03/17/2008 to discuss the difference between the Studer's protocol and the claimed protocols. Appellants also state that the examiner addressed the original limitation "at least 7 days" in original claim 4 because the steps and stages in the Studer's protocols require at least 6-9 days. Based on the office action dated 12/24/08, Appellants acknowledge that it is obvious to sequentially add growth factors because at the end, the culture medium would inherently encompass added growth factors. Based on the office action dated 10/20/09, Appellants argue that the examiner provides no evidentiary support that modifying the Studer or Lee protocol to the claimed protocol would have been expected to generate dopaminergic neurons. Appellants' arguments have been considered but they are not persuasive.

In response, the examiner asserts that the instant claims are rejected under 103 (a) (obviousness) not an anticipatory rejection (102). The rejections are maintained for the reasons as set forth above at section **(9) Grounds of Rejection**. In addition, Appellants' summary has been noted. However, the appealed claims have been amended, which are different from original claims. Appellants cannot use the instant

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claims to argue against the responses from different office actions because these different office actions were used to address different claim limitations in different claim amendment.

On p. 13-15 of appeal brief, Appellant argues that one would not achieve the claimed invention by combining the cited reference because the procedures of Lee do not contain all of the elements of the claims. Appellant argues that none of the disclosures meet the limitation of step (d) co-culturing with astrocytes. Appellants argue that although Studer teaches glial cells, glial cells may or may not be astrocytes. In addition, Appellants argue that there is no teaching that any cell is incubated with an astrocyte for 7 day because any astrocyte would be an end product of the protocol, which is produced by the stage V conditions. In addition, Appellants argue that even the culture of Lee contains both neurons and astrocytes, the astrocytes are the end product of the stage V and there is no disclosure of culturing together for 7 days. Appellants' arguments have been considered but they are not persuasive.

In response, although Studer does not explicitly teach co-culturing stem cells that are treated with the claimed growth factors with astrocytes, the cell culture of Studer is a cell mixture, which encompasses astrocytes. Although Studer does not explicitly teach glial cells including astrocytes in stem cell culture, the astrocytes encompassed with in the culture is evidenced by the teaching of Lee. Lee teaches a method of expanding embryonic stem (ES) cells and the CNS precursor cells in a DMEM plus F12 medium comprising bFGF, FGF8, Shh, N2 supplement, insulin, transferrin, selenite, putrescine,

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and progesterone as recited in instant claims 1 and 13 (see p.9, [0123]-[0126], in particular). Lee teaches differentiation of cultured embryonic stem cells into neurons using the same medium in presence of ascorbic acid, and the differentiation of stem cells encompasses 3% astrocytes. The presence of astrocytes in the culture of stem cells that are differentiated into neurons meets the limitation of "comprising co-culture astrocytes" as recited in instant claims 1 and 13. Thus, the presence of glial cells including astrocytes meets the limitation "co-culturing cells with astrocytes". It is known in the art that neuronal cultures require astrocytes to maintain and support neuronal survival and differentiation because the mixture of neuronal stem or progenitor cells that are capable of differentiating into neurons also encompass cells that are capable of developing or differentiating into astrocytes. Further, it is known in the art and it is a standard procedure to culture neuronal progenitor cells or neural stem cells with astrocytes or an astrocyte feeder layer to maintain or enhance neuronal survival as evidenced by Walsh et al. (Neurosci. Lett 1992. 138: 103, abstract, cited in office action dated 12/24/08, p. 8). Thus, it is obvious to co-culture stem cells that have been treated with bFGF, FGF8, Shh and BDNF with astrocytes to induce neuronal differentiation.

In addition, although Studer does not explicitly teach at least 7 days for each step as recited in instant claims 1 and 13, each step and each stage of the culture conditions of Studer require 6-9 days and the whole culture procedures take more than one month (see p.27-29, in particular). Thus, the culturing steps and conditions are within the limitation of the instant claims 1 and 13. Further, Lee teaches that the CNS precursor cells (including CNS stem cells and embryoid cells/neurospheres) are expanded in the

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CNS proliferation medium in the presence of bFGF or EGF for about 6 to 7 days (see p. 9, [0123]-[0124], in particular). Lee also teaches that the culture medium may also be supplemented with SHH and FGF8 because SHH and FGF8 are more effective to increase the generation of dopaminergic neurons (see p.9, [0125], in particular). Moreover, Lee teaches that neuronal differentiation is induced by withdrawal of at least one neurological agent, such as bFGF in the culture medium in the presence of the factors to enhance the generation of dopaminergic neurons for five to 6 days (see p. 9, [0128]; p. 14, example 5, in particular). Although Lee does not explicitly teach at least 7 days for each step as recited in instant claims 1 and 13, the complete culture procedures of Lee take more than one month (see p.8, [0111], in particular).

It would have been obvious to one of ordinary skill in the art at the time the instant invention was made to incorporate the teachings of Lee to the method of Studer to modify and optimize the culture conditions to induce neuronal differentiation from stem cells. The person of ordinary skill in the art would have been motivated to do so with an expectation of success because both Lee and Studer teach the use of different growth factors separately and the use of different growth factors can enhance different cell populations at different stages. For example, Lee and Studer teach that bFGF can be used to expand ES cells, and FGF8 and SHH can be used to increase the generation of dopaminergic neurons. In addition, the culture medium in the presence of bFGF and in the absence of FGF8 and SHH can increase astrocyte generation. Further, the culture medium in the presence of BDNF and in the absence of FGF8 and SHH can induce neuronal differentiation. Thus, it would have been obvious to add

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different growth factors sequentially. The person would have been motivated to do so with an expectation of success because each different growth factor can increase specific type cell populations and the use of the combination of the claimed growth factors have been shown to successfully induce neuronal differentiation from stem cells. The combined teachings of Studer and Lee has taught that the addition of bFGF is to induce ES cell expansion and can enhance astrocyte generation for neuronal survival, the addition of FGF8 and SHH to the expanded ES cells after the exposure of bFGF can enhance the generation of dopaminergic and serotonergic neurons, and the addition of BDNF after the exposure of FGF8 and SHH can induce neuronal differentiation.

Moreover, although Studer and Lee do not explicitly teach at least 7 days for each step, the claimed procedures and incubation time for each step are obvious over the cited reference because the incubation time for each step is within or overlaps with the claimed incubation time. In addition, as mentioned above, it is known in the art that neural cells (neural progenitor/stem cells) co-cultured with astrocytes or on an astrocyte feeder layer can enhance neuronal survival and differentiation. Thus, it has been obvious to combine the teachings of Studer and Lee to achieve and practice the claimed invention because the results of dopaminergic and serotonergic neuronal differentiation are expected.

On p. 16-17 of the appeal brief, Appellants argue that there is no motivation or reasonable expectation of success. Appellants argue one would not have expected the same results of adding growth factors sequentially at nest-positive (stage III) cells as

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simultaneous exposure. Appellants argue that the phenotypes produced by exposing the cells to the growth factors sequentially for the 7 day period would not be predictably same as those from simultaneous exposure and submit Dr. Verfaillie's declaration and KSR International v. Teleflex Inc. in support of the arguments. On p. 17 of the appeal brief, Appellants argue that Dr. Verfaillie further cites the reference of Snykers et al. in the declaration to explain Appellants' position that the phenotypes of the cells while exposed to growth factors sequentially cannot be predicted based on the results of simultaneous exposure to the same factors. Appellants' arguments have been considered but they are not persuasive.

In response, although the instant method recites adding bFGF, FGF8, SHH and BDNF sequentially, the claimed method is only directed to inducing neuronal differentiation and is not directed to differentiation into specifically defined proportions of specific types of neurons. Note that the claimed method and the cited references are directed to the same goal (neuronal differentiation from stem cells) using the same materials (bFGF, FGF8, SHH and BDNF). The claimed method is directed to inducing neuronal differentiation using the same growth factors (bFGF, FGF8, SHH and BDNF) and the same ES cells and similar culture duration, which are taught by the cited references. Although the claimed method alters the way of adding growth factors in the methods of Studer and Lee, and phenotypical cell types may have different proportions during the recited culturing procedures, at the end of the steps, the result of neuronal differentiation from stem cells including dopaminergic, serotonergic and GABAergic

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neurons is expected as taught by Studer and Lee (see p.9, [0125], p. 9, [0128]; p. 14, example 5, in particular).

In addition, given the examination guidelines for determining obviousness under 35 U.S.C. 103 in view of the Supreme Court decision in *KSR International Co. V. Teleflex Inc.* 82 USPQ2d 1385 (2007) and the Examination Guidelines set forth in the Federal Register (Vol. 72, No. 195, October 10, 2007) and incorporated recently into the MPEP (Revision 6, September 2007), the following rationales to support rejection under 35 U.S.C. 103(a) are noted:

A) Combining prior art elements according to known methods to yield predictable results.

B) Simple substitution of one known element for another to obtain predictable results.

C) Use of known technique to improve similar products in the same way.

D) Applying known technique to a known product ready for improvement to yield predictable results.

E) "Obvious to try" --- choosing from a finite number of identified, predictable solutions, with a reasonable expectation of success.

F) Some teachings, suggestion, or motivation in the prior art that would lead to one of ordinary skill to modify the prior art reference to arrive at the claimed invention.

In this case, the neuronal differentiation results derived from sequential addition of growth factors are expected because both the claimed method and the methods of Studer and Lee are directed to induction of neuronal differentiation of stem cells (i.e. same goal using same materials). The claimed method is a simple substitution of the step of simultaneous exposure of cells to the same growth with the step of sequential addition of the same growth factors. In addition, it is also obvious to try and modify the order of adding the same growth factors because the stem cells are also expected to be differentiated into neuronal after sequential treatment of the same growth factors. Note

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that obviousness is not the result of a rigid formula disassociated from the consideration of the facts of a case. Indeed, the common sense of those skilled in the art demonstrates why some combinations would have been obvious where others would not. See *KSR International Co. V. Teleflex Inc.* 82 USPQ2d 1385 (2007). From the combined teachings of the references, it is apparent that one of ordinary skill in the art would have had a reasonable expectation of success in producing the claimed invention.

On p. 18-19 of appeal brief, Appellants argue that it is an erroneous reason to dismiss Dr. Vefaille's declaration because the examiner provides no scientific evidence to rebut the Dr. Vefaille's declaration. In addition, Appellants argue that it is erroneous to dismiss the Dr. Vefaille's declaration because a side-by-side comparison is not required. Appellants further argue that the citation of the Snyder reference is relevant to Dr. Vefaille's declaration because the cells don't need to be the same, and the citation of Snyder is to illustrate the principle that exposure of a progenitor cell to the same factors but in different sequence, will not necessarily produce the same differentiated end product. Appellants' arguments have been considered but they are not persuasive.

In response, contrary to Appellants' arguments, the Dr. Vefaille's declaration has been considered but the declaration is insufficient to overcome the rejection because the supporting evidence of the Snyder reference is not relevant to Dr. Vefaille's statement in the declaration. In addition, contrary to Appellants' arguments, the examiner does provide scientific reasons to rebut Dr. Vefaille's declaration. In

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particular, the examiner states that Appellants' arguments are contradictory to Appellants' own position as stated in Dr. Verfaillie's declaration. Dr. Verfaillie's declaration states that different phenotypes of neural cells will respond to growth factors differently. However, the instant claims are not directed to different phenotypes of neural cells. Rather, the cells used in the instant claims are stem cells, which are identical to the cells used in the Studer's or Lee's method. The claimed method is directed to inducing neuronal differentiation from stem cells. In addition, if the statement in the declaration is true, the evidence based on the cited reference (Snykers et al. Toxicological science. 2006, 94: 330-341) cannot be applied to the instant case because the cells taught by Snykers are directed to hepatic cells, which are different cell types from the instant cells. The hepatic cells will act differently from neural stem cells in response to growth factors and thus will differentiate into different cells. Thus, the effects or results of hepatic cells cannot be used to compare with stem cells that are capable of differentiating into neurons because different cell types have different cellular components, cell surface receptors and biological characteristics. When the hepatic cells and neural stem cells are exposed to the same growth factors, they will respond to growth factors differently. However, if the cells are the same type of the cells, they will respond to the same growth factors similarly because they have similar cellular components, cell surface receptors and biological characteristics. Thus, the evidence cited in the Dr. Verfaillie's declaration is not relevant to the instant applications and is insufficient to overcome the rejection.

In addition, Appellants fail to provide data to demonstrate that the end product from the instant claims is different from the end product of Studer and Lee. Appellants fail to show the end product will be different when both the instant and the art methods are directed to the same goal (i.e. induction of neuronal differentiation) and use the same materials (stem cells and same growth factors). Note that both of the claimed method and the methods of Studer and Lee are directed to inducing neuronal differentiation from stem cells and use the same materials (stem cells and growth factors). Although the claimed method is directed to adding growth factors (same growth factors) sequentially to stem cells, the end result is directed to neuronal differentiation. Note that the instant case is based on the same known materials (the functions and the known effects of each growth factor on stem cells) and known culture protocols for neuronal differentiation from stem cells to modify and optimize the culture protocols. Thus, the results of neuronal differentiation from stem cells are expected. It is noted that the instant claims are not directed to generation of different proportions of different neuronal cell types. If Appellants' position is that the results from the claimed method (i.e. sequential exposure to the same growth factors) would be different from the results generated from simultaneous exposure to the same factors, Appellants are required to provide data to demonstrate the difference between the Studer and Lee's methods and the claimed method to distinguish different end results as claimed.

On p. 20 of the appeal brief, Appellants argue that Lee teaches away from adding the Shh/FGF at a later stage than the bFGF. Appellants argue that when bFGF

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was added prior to FGF8 and Shh, it is ineffective to produce dopaminergic neurons.

Appellants' arguments have been fully considered but they are not persuasive.

In response, first, the claimed method is not directed to specifically induce differentiation of dopaminergic neurons. Second, contrary to Appellants' arguments, although the instant method recites adding bFGF, FGF8, SHH and BDNF sequentially, the claimed method is only directed to inducing neuronal differentiation. The claimed method is not directed to specific differentiation into specific defined proportions of specific types of neurons. It is noted that the claimed method and the cited references are directed to the same goal using the same materials. The claimed method is directed to inducing neuronal differentiation using the same growth factors (bFGF, FGF8, SHH and BDNF) and the same ES cells, which are taught by the cited references. Although the claimed method alters the way of adding growth factors, and phenotypical cell types may have different proportions during the recited culturing procedures, the end result of neuronal differentiation is expected to generate neurons including dopaminergic, serotonergic and GABAergic neurons.

In addition, contrary to Appellants' arguments, the examiner asserts that Lee does not teach away from adding the Shh/FGF8 at a later stage than the bFGF. Lee only teaches that addition of SHH and FGF8 at the early stages is less effective (see p. 9, [0126], in particular), which provides motivation to add SHH and FGF8 after addition of other growth factor that induces proliferation such as bFGF. Furthermore, Lee teaches that the CNS precursor cells (including CNS stem cells and embryoid cells/neurospheres) are expanded in the CNS proliferation medium in the presence of

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bFGF or EGF for about 6 to 7 days (see p. 9, [0123]-[0124], in particular). Lee also teaches that the culture medium may also be supplemented with SHH and FGF8 because SHH and FGF8 are more effective to increase the generation of dopaminergic neurons (see p.9, [0125], in particular). Moreover, Lee teaches that neuronal differentiation is induced by withdrawal of at least one neurological agent, such as bFGF in the culture medium in the presence of the factors to enhance the generation of dopaminergic neurons for five to 6 days (see p. 9, [0128]; p. 14, example 5, in particular).

Furthermore, Studer teaches that in order to generate astrocytes, before adding SHH and FGF8 into the culture medium, the ES cells are cultured and proliferate in a culture medium in the presence of bFGF (see p. 5, paragraphs 16-17; p. 26, paragraph 78, in particular). Studer teaches that to enhance dopaminergic and serotonergic neurons, ES cells are cultured in the proliferation culture medium in the presence of FGF8 and SHH for 6-9 days (see p. 28, paragraph 85; p. 29, paragraph 86, in particular). Studer also teaches that expanded ES cells from stage IV are induced to neuronal differentiation in the culture medium in the presence of BDNF and in the absence of SHH and FGF8 for 4-10 days (see p. 26, paragraph, 78; p.29, paragraph 87, in particular).

Both Lee and Studer do teach the use of different growth factors separately because each different growth factor can enhance different cell populations. For example, Lee and Studer teach that bFGF can be used to expand ES cells, and FGF8 and SHH can be used to increase the generation of dopaminergic neurons. In addition,

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the culture medium in the presence of bFGF and in the absence of FGF8 and SHH can increase astrocyte generation. Further, the culture medium in the presence of BDNF and in the absence of FGF8 and SHH can induce neuronal differentiation. Thus, it would have been obvious to add different growth factors sequentially. The person would have been motivated to do so with an expectation of success because each different growth factor can increase specific type cell populations. In this case, the addition of bFGF is to induce ES cell expansion and can enhance astrocyte generation for neuronal survival. The addition of FGF8 and SHH to the expanded ES cells after the exposure of bFGF can enhance the generation of dopaminergic and serotonergic neurons. The addition of BDNF after the exposure of FGF8 and SHH can induce neuronal differentiation.

Taken together, the claimed method of sequentially adding growth factors to ES cells is obvious over the cited references because Lee and Studer do provide a motivation and an expectation of success to add growth factors sequentially. Lee and Studer teach that each different growth factor used separately can enhance different cell populations. For example, ES cells incubated with bFGF alone with no FGF8 and SHH will increase ES cell proliferation and increase astrocyte generation, and astrocytes have been shown to enhance neuronal survival. In addition, expanded ES cells incubated with FGF8 and SHH will increase the generation of dopaminergic and serotonergic neuronal lineage. Finally, ES cells treated with FGF8 and SHH and further incubated with BDNF without bFGF or FGF8 and SHH can be induced into dopaminergic neurons. Thus, a skilled artisan would have been motivated and would

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have expected success to add growth factors sequentially to induce neuronal populations.

B. On p. 21 of the appeal brief, Appellants argue that the examiner does not explain how Song would apply to claims 1 and 13. Appellants argue that Song does not cure the deficiencies of Studer and Lee. Appellants' arguments have been fully considered but they are not persuasive.

In response, the citation of Song is to support the original claims that are directed stem cells derived from different tissue including multipotent progenitor cells, bone marrow and blood. Note that Song was cited for the subject matter of differentiating bone marrow and umbilical cord blood cells into neural progenitor cells and neurons. Appellants cannot show non-obviousness by attacking each individual reference when the rejection is based on the teachings of the combined references.

Contrary to Applicant's arguments, for the reasons as set forth above, Studer and Lee do render the claimed invention obvious. However, Studer and Lee do not teach stem cells from other tissue or bone marrow. Although Studer and Lee do not teach multipotent adult progenitor cells and stem cells from bone marrow, Song et al. teach a method of culturing and differentiating bone marrow and umbilical cord blood cells into neural progenitor cells and neurons in a DMEM/F12 medium comprising FGF-2/bFGF, EGF, transferrin, insulin, putrescine, progesterone, selenium, trans-retinoic acid, BDNF and NGF (see p. 80, in particular). Song et al. also teach culturing human and mouse bone marrow and human umbilical cord cultures (p. 82-83). The bone marrow and

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umbilical cord blood cells encompass stem cells, and nonhematopoietic progenitor cells from bone marrow are mesenchymal stem cells or bone marrow stromal cells as taught by Song et al. (p.79, in particular). Thus, it is obvious to differentiate stem cells that are derived from multipotent adult progenitor cells (MAPCs) and bone marrow into neurons by using the culture conditions of Studer and Lee.

(11) Related Proceeding(s) Appendix

No decision rendered by a court or the Board is identified by the examiner in the Related Appeals and Interferences section of this examiner's answer.

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For the above reasons, it is believed that the rejections should be sustained.

Respectfully submitted,

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